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Notes on the Manner of Growth of the Cell Wall.

By Emily L. Gregory.

(Plate CIX).

The question of the manner of growth of the cell wall in the vegetable kingdom has not yet been satisfactorily answered. If we accept the theory of Nægeli in respect to the crystalline form of the micellæ composing it, and suppose the first or primary wall to be formed according to the laws governing the arrangement of such figures, when lying free in a fluid substance, the question then arises, how does this wall grow in surface and in thickness? Nægeli says in his work on the growth of the starch grain, that before this question can be answered many facts must be observed and registered.

The object of the present brief paper is to record one or two facts which appear to be connected with this question. It is not expedient therefore to give a resume of the work already done and the facts discovered in this field since the suggestion of Nægeli. An article written by Krabbe in Pringsheim's Jahrbuch in 1887 contains an account of some very interesting observations and experiments, and in this paper the author claims to have proven one or two points in this connection which have hitherto lacked positive demonstration. Without taking space here to review this whole article, which is a long and valuable contribution to the literature on this subject, I may be pardoned for repeating some parts of it here.

In the study of stratification and striation in the walls of bast and thick-walled libriform cells, Krabbe claims that the observations preceding his own have been made on longitudinal sections, and therefore the results are not always reliable.

A stratified wall consists of several layers which appear to be quite distinct from each other when seen on the cross-section. These layers often appear striated, or as having stripings running more or less obliquely through them which on the cross-section show like narrow lines. Krabbe used cross-sections of different stems, *Linum, Urtica* and others, and by an ingenious method of focusing by which the lines, according to their relative position in the layer, were made to recede from or approach each other, claims to have proven that the process of intussusception could not have taken place in the growth in thickness of the entire wall. That is, he claims to have shown that the growth in thickness of each one of these lamellæ or layers was entirely separate from that of the others, and that no interchange of micellæ from one layer to another could have taken place.

The result of this study, ingenious as it was, led to no definite results as to the actual manner of increase of thickness in a wall already formed. Admitting that the two lamellæ are entirely independent of each other, each containing its own micellæ from the first, the question still remains, how did they reach their present thickness? He agrees with all the recent investigators on this subject, that in many cases a so-called new formation of wall occurs. To explain this we will suppose a cell with moderately thickened walls; this when about to add to its thickness throws out a new wall which eventually sets itself firmly on the old one so as to become part and parcel of it. This building a new wall by the protoplasm of an already enclosed cell is a process quite lately discovered and in German is known as "neubildung."

In another portion of his work he claims to have reached more satisfactory results; in fact to have actually proven that intussusception does take place in the increase of thickness in certain walls. In certain plants of the families Asclepiadaceæ and Apocynaceæ there occurs a process of widening or bulging out of the walls of the bast cells in a very singular fashion, and afterward a cellulose wall may be built across so as to form separate cells in the once single bast fiber. Now as to the growth of these widenings it is plain that the number of micellæ composing the growing cell wall must have increased considerably. The question how this increase is managed Krabbe answers by saying that it

can be in no other way than by intussusception. He gives elaborate arguments to prove that the turgor could not be sufficient to account for it on the principal of stretching, says that a pressure of from 3,750 to 5,000 atmospheres must be assumed in order to produce such a result, and that it is impossible to premise a pressure of more than 1,000 atmospheres in a cell. Other serious objections are given, and finally, because Strasburger denies that a cell can grow in surface by any other process than by stretching, he says, admitting it to have grown by stretching, we must then expect it to be proportionately thinner, but such is not the case. Measurements are given showing the wall to be of uniform thickness. Therefore it is proven that these local thickenings in the bast cells of these plants must be the result of the process of intussusception.

Then he gives his reasons for concluding that in most cases of thickened walls showing striation the process of new formation has taken place, a wall being built and then added to the previous one. He says, admitting this latter statement proven, " Still the real question is not touched, for who can say how this new wall increased in thickness in its process of growth, whether by apposition or intussusception." In the case of the growth of the widened places in the bast fibers, however, a definite point is gained; if the proofs are satisfactory then we may hold that the theory of growth in thickness by intussusception in some cases is established.

While the writer was studying the development of wings on the stems of the genus *Euonymus* one or two instances of peculiar growth were noticed. About eighteen different species or varieties of this genus were studied, but only one of them showed this peculiarity in wall formation. This was *Euonymus angustifolia*, var. *purpurea*; the specimens were obtained from the nursery of Thomas Meehan, near Philadelphia. This species has the usual four-angled stem with slight but perfectly developed wings along these angles. It will be necessary here to repeat a little from the work on cork wings referred to above: " A cross-section at the distance of ½ centimeter from the growing tip of stem shows the tissues of the rind in a formative stage. The outer wall of the epidermal cells is covered by a thin cuticle, the cells themselves

are large, nearly isodiametric, and with thin walls; below the epidermis are two layers of thin-walled cells without chlorophyll, then four or five layers of parenchymatic chlorophyll-holding cells, and then below these again several layers of colorless cells." At the corners of the stem the rows of chlorophyll-holding cells are broken by a cluster of collenchymatic colorless cells. In a later stage of development the central cells of this cluster form the string of bast fibers which are pushed out by the growing wing. It is with the growth and development of these bast fibers that a point of interest occurs. A section cut at the distance of ½ centimeter from the growing tip (Plate CIX. fig. 1.) shows this little central cluster of cells, with thick walls and small lumen; now a section at three centimeters from the tip shows that several important changes have occurred. The two cylinders of colorless cells now begin to take on a collenchymatic appearance, the walls having thickened considerably. The cells of the epidermis are in a state of rapid growth and division, new cells being added to the outer cylinder whose walls rapidly thicken, as may be seen by sections at the distance of five and seven centimeters from the tip. The cells in the center of the group in the corners are seen to have increased in diameter while at the same time their walls are much thinner.

Now if we follow this same set of cells, cutting at various distances below this, we find them with extremely thick walls, so that only a small point appears as the lumen. Their diameter is about the same as at the distance of three centimeters, but in whatever way the additional thickness has been acquired the original wall as seen at the latter distance is perfectly evident as a dark line marking the outline of each cell.

Here, if we accept Strasburger's theory, that growth in surface takes place by stretching, we have an apparently consistent explanation of the processes which have taken place. The thick walled cells in fig. 1 c., have changed to thin-walled wider lumened cells as seen in fig. 2, by using up the material already in their walls. By this process of stretching the diameter is increased until the proper size for the bast fibre is obtained. Then by a succeeding process this thin wall is added to until hardly a trace of cavity remains. If, on the other hand, as in the case of

the bast fibres described by Krabbe, it can be proven that the turgor cannot be raised sufficiently to produce this effect of stretching, the only other explanation possible is that the walls of the cells in question have undergone the following cycle of changes: Their thin walls produced by the original division of the cells composing them have been thickened so they have the appearance as shown in fig. 1; then the matter composing these walls has been partially absorbed till only the light wall remains as seen in figure 2; this must also have increased in surface to allow for the increased diameter as seen here. Then later on a new process of thickening has taken place, by which the wall is made much thicker then before. It must be noticed here that there is not the same difficulty in the way of the wall stretching out till the diameter required is reached as in the case described by Krabbe. Here the young walls are in their early stage of growth and there is every probability that a much smaller pressure would serve to extend them than in case of the bast fibers of an older tissue.

It is true that the simple fact that one explanation appears more plausible than another, is not at all of the nature of proof in scientific research. At the same time the rapid succession of changes noticed in the thickness of the walls of this tissue seems worthy of record as a fact bearing on this subject.

Another peculiarity still more striking than this occurs in the course of the rapid growth and division of the epidermal cells between the wings. As before stated, these originate from a phellogen layer extending not around the stem, but only for a short distance around and beyond the little cluster of bast cells, the wing shoving these out as it develops. In this variety the wing never acquires a very great depth, but is of width enough to materially aid in the enlargement of the circumference, while the spaces between are thus enabled to assimilate. It is only, therefore, on the spaces between these wings that the epidermal cells retain their character and divide, forming new ones. This process does not result in the formation of corky layers, composing the tissue known as periderm, but they form an additional support to the outer collenchymatic cylinder, which at first is only two layers in thickness. By means of these additional cells the

number is increased often to six or seven layers. In regard to these collenchymatic layers being necessary to support the stem, it may be mentioned in passing that this stem is one of the class having no bast fibers in its outer or rind portion.

The real periderm consists of the wings until the stem gets older—two or three years in some instances—when the assimilating surface is no longer needed, the bands of phellogen cells extend themselves until a circle is formed around the entire stem.

Now it is with the stem before the wings form and for some time after this, that we have to do. In order to allow the cells under the epidermis to fulfill their office of assimilating, the epidermal cells themselves must remain intact and perform their office as do the epidermal cells of the leaf. It is necessary, therefore, that they divide and increase rapidly in order to keep pace with the increasing growth in diameter. This in itself presents no difficulty as long as the cell contents are in working order and the cell wall is of pure cellulose, so that it can change in any way to enable it to increase in thickness or in surface; this process of growth and cell division is normal to the plant.

There is, however, one difficulty in this for the epidermal cells, which is not found usually elsewhere. This is the heavy upper or outside wall, the cellulose of which turns to suberin or cutin at a very early stage of the growth of the stem. One of the characteristics of suberin is that it is nearly impervious to air. It is also supposed to be much less liable to change of structure than cellulose. By this is meant that the micellæ composing it do not so readily change place, therefore change in form or structure is more difficult in case of suberized wall than that consisting of pure cellulose. In other words a cell with purely cellulose wall with living contents is always liable to changes by means of growth, while the wall turned to suberin or lignin is a sign of completed growth, fixedness of form.

Now in our *Euonymus* stem at a distance of one-half centimeter from the tip these epidermal cells are found partly suberized as to their upper wall, this wall is thicker than the other, but the shape of the cell is nearly cubical. At a distance of three centimeters from the tip and from this on till the stem has acquired some age, the outer walls of the epidermal cells are very

thick and strongly cuticularized. Instead of stretching across the cell at right angles with the radial walls, and parallel with the lower tangential walls, they are strongly inclined upward, many making a sharp angle. Here, where the wall is so plainly cuticularized, the process of division is rapidly going on; new walls are seen in both directions, tangential and radial. But, as before stated, the tangential divisions are limited for the most part to four or five layers of cells, forming the collenchymatic cylinder outside the palisade cells. The radial divisions, of course, keep pace with the increasing circumference, and the outer tangential wall must increase at a rapid rate; it must increase not only in surface, but it does keep pace in thickness, for no section can be made which does not show all the cells of the epidermis fitted with outer walls of uniform thickness.

Now if we turn to the sections studied, we shall see a peculiar development which must in some way serve to aid in this series of rapid changes. We have before mentioned a curvature in the wall in nearly all cells in the process of division (which process is detected by the thinness of the new wall). Besides the thin new wall just forming, there is another element which, so far as I know, is peculiar to this variety. This is a sphere or spherical body consisting of partly cuticularized cellulose, extending from the outer wall down into the cell, to which in all cases is attached the new wall. That is to say, this spherical body projecting down into the cell was never found unless below it was the new radial wall which was just forming to increase the number of cells in the circumference. New cells were sometimes seen without the sphere, but the sphere is never found without the wall. Now by a study of the surface of these cells the following facts are recognized:

These spheres are found principally or more frequently on the tangential walls of the grown epidermal cells. At the stage where they are most numerous, these cells have a long diameter parallel with the axis of the stem. The tangential diameter is the one which must enlarge so rapidly to keep pace with the increase in circumference, and these bodies occupy such a position as to be readily made use of in the new outer wall.

If we suppose this to be the function of these bodies, the

question at once arises. By what process is their substance taken up into the wall? There is little probability that the latter stretches out so as to use up this material, as in the case of the cellulose ring in the *Œdogonium* cell. The shape is not adapted to this purpose, therefore a process similar to that of intussusception must take place. The assumption that this is the function of these globular bodies, and that they are merely reserve stores enabling the outer wall of the epidermis to preserve its uniform thickness, we admit, rests on very slight proofs. There are, however, several indications which point in this direction.

Chemically, they agree perfectly with the outer wall from which they extend. The greater portion of this wall consists of cutin in its early stages, a layer of pure cellulose is found to extend along the inner surface connecting with the side walls, and this cellulose extends entirely around the sphere. In fact, it is as though a little bag of cellulose membrane were sunken in here and filled with cuticularized substance. There is no question as to its chemical nature, as it was repeatedly and thoroughly tested. Again its connection with the new young wall is suggestive of this function. Except for this, its appearance is quite similar to the incrustations of calcium carbonate sometimes found in the epidermal cells of *Ficus.** However, cystoliths do not occupy such a position in reference to new walls, neither do they consist of cuticularized cellulose. Another reason for the assumption is the lack of these bodies in the epidermis of the grown stem, or that portion where the new periderm is about to form and the epidermis is to be thrown off.

EXPLANATION OF PLATE CIX.

Figures 1, 2 and 3 represent cross-sections of stem of *Euonymus angustifolia*, var. *purpurea*. They include only a corner in each instance, containing a bundle of bast cells, which appears in fig. 3 in a mature or finished condition.

Fig. 1 shows a section cut one-half centimeter below the tip of the stem, fig. 2 three centimeters, and fig. 3 twenty-five centimeters from this tip. In each of these figures *a* marks epidermal tissue, *b* the subepidermal or supporting-cells, and *d* those holding chlorophyll.

In fig. 1, *c* marks the collenchymatic cells of the corner, which later on develop into bast fibers. In fig. 2, these cells *c* are thin-walled, and have a larger lumen. In fig. 3 the same letter marks the cluster now completed.

Fig. 4 represents the epidermal layer as seen from above—distance from tip of

*See Haberlandt Physiologische Pflanzen Anatomie, page 340.

stem about twenty-five centimeters : *a* marks the spherical projection into the cells.

Fig. 5 shows cross-section through epidermal and subepidermal layers at the same distance from tip as in fig. 4 ; *a* shows here the spherical projection.

Fig. 6.—Same as fig. 5, only a longitudinal section.

Fig. 7.—Cross-section showing epidermal cells, cut one-half centimeter from tip ; *a* here shows beginning of a stoma.

A Further Enumeration of some Lichens of the United States.

BY DR. JOHN W. ECKFELDT.

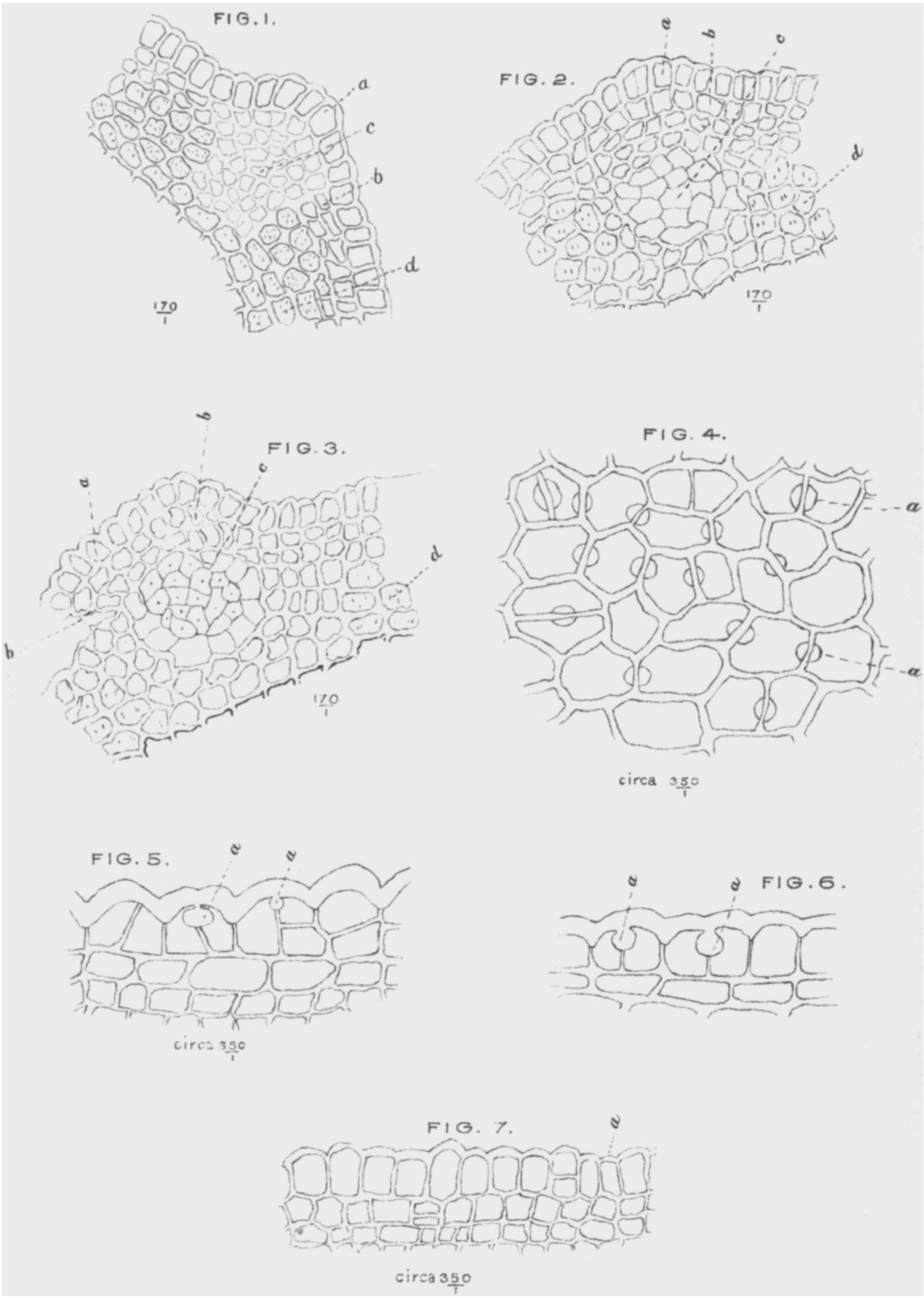
The following account includes a few lichens heretofore but little known as occurring within the limits of the United States, and as two of these species have been described elsewhere, and as they are comparatively new or uncommon to the region specified, I deem it wise to call further attention to them. It will be further noticed that I have included three new species; these were collected during the winter of 1887, and were kindly identified by Dr. W. Nylander, whose descriptions I here include. Formerly the other plants were recorded as occurring only in the island of Cuba.

LECIDEA (BIATORA) MESOPHŒA, Nyl. spec. nova. Thallus cinerascens vel cinereo-virescens, subleprosus, tenuis, indeterminatus; apothecia livido-nigricantia (latit circiter 0.5 millim.), lecanoroidea obmarginem conceptacularem albicantem (vix prominulum), intus strato media obscurato; sporæ 8-næ, fusiformes, 3-septatæ, longit 0.011-15, crassit 0.004 millim., epithecium incolor, paraphyses non discretæ, hypothecium supra fuscum infraque incolor. Iodo gelatina hymenialis cœruliscent, dein fulvo rubescens.

Species videtur e stirpe *Lecidia* (*Biat. violaceæ* ab. omnibus distincta apotheciis intus albis striato hypotheciali superiori infuscato. Tuckerman in hb. eam dexit *Platygrapham* et adert quædam facies *Platygraphæ prominulæ*, Nyl. Enumer. p. 131 Guyanensis quae vero distat hypothecio fusco et alus notis varus.

A rather rare and well marked species found throughout Florida, but recently collected at Jacksonville on barks at the base of various trees, by Mr. Calkins. This plant evidently belongs to the more tropical regions of America, and may be found by careful search in the island of Cuba.

LECIDEA (BIATORA) ORPHNŒA, Tuck. Proceed. 1864 p. 274. Tuck. synop. pars. 2, p. 157. Thallus obscure rufescens, mi-



GREGORY ON THE MANNER OF GROWTH OF THE CELL-WALL.